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(54) Title: COMPOSITIONS FOR THE REMOVAL OF DENTAL PLAQUE

(57) Abstract

The present invention relates to oral care compositions and products comprising a dextranase and a mutanase, and optionally other enzymes.

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Title: Compositions for the removal of dental plaque

FIELD OF THE INVENTION

The present invention relates to oral care compositions and products comprising a dextranase and a mutanase, and optionally other enzymes.

The invention also relates to the use of the composition and product of the invention for the removal of dental plaque and preventing the formation of dental plaque.

BACKGROUND OF THE INVENTION

The formation of dental plaque leads to dental caries, gingival inflammation, periodontal disease, and eventually tooth loss. Dental plaque is a mixture of bacteria, epithelial cells, leukocytes, macrophages, and other oral exudate. Said bacteria produce highly branched polysaccharides which together with microorganisms from the oral cavity form an adhesive matrix for the continued proliferation of plaque.

As plaque continues to accumulate rock hard white or yellowish deposits arise. These deposits are called calcified plaque, calculus or tartar, and are formed in the saliva from plaque and minerals, such as in particular calcium.

Oral polysaccharides

Oral polysaccharides are produced from sucrose introduced into the mouth, *e.g.* as a food or beverage constituent, by the action of cariogenic microorganisms, such as *Streptococcus mutans* or *Streptococcus sanguis*, growing in the oral cavity.

Said oral polysaccharides comprise water-soluble dextran, having large portions of a-1,6 glucosidic linkage, and a major component of water-insoluble extracellular polysaccharides called "mutan" comprised of a backbone with a-1,3-glycosidic linkages and branches with a-1,6-glycosidic linkages.

Mutan bind to hydroxyapatite (constituting the hard outer porous layer of the teeth) and to acceptor proteins on the

cell surface of said cariogenic bacteria adhering to the teeth surface.

Mutanase

Mutanases are a-1,3-glucanases (also known as a-1,3-glucanohydrolases) which degrade the a-1,3-glycosidic linkages in mutan. Mutanases have been described from two species of *Trichoderma* (Hasegawa et al., (1969), Journal of Biological Chemistry **244**, p. 5460-5470; Guggenheim and Haller, (1972), Journal of Dental Research **51**, p. 394-402), from a strain of *Streptomyces* (Takehara et al., (1981), Journal of Bacteriology **145**, p. 729-735), *Cladosporium resinae* (Hare et al. (1978), Carbohydrate Research **66**, p. 245-264), *Pseudomonas* sp. (US patent no. 4,438,093), *Flavobacterium* sp. (JP 77038113), *Bacillus circulanse* (JP 63301788) and *Aspergillus* sp.. A mutanase gene from *Trichoderma harzianum* has been cloned and sequenced (Japanese Patent No. 4-58889/A).

Dextranase

Dextranases are a-1,6-glucanases (also known as 1,6-a-D-glucan 6 glucanohydrolases) which degrade the a-1,6-glycosidic linkages in dextran. Several microorganisms are capable of producing dextranases, among them fungi of *Penicillium, Paecilomyces, Aspergillus, Fusarium, Spicaria, Verticillium, Helminthosporium* and the *Chaetomium* genera; bacteria of the genera *Lactobacillus, Streptococcus, Cellvibrio, Cytophaga, Brevibacterium, Pseudomonas, Corynebacterium, Arthrobacter* and *Flavobacterium* and yeasts such as *Lipomyces starkeyi*.

Commercially available products include Dextranase 50 L from Novo Nordisk A/S produced by fermentation of strains of *Penicillium lilacium*. Dextranase 50 L is used in the sugar industry to break down dextran in raw sugar juice or syrup.

To be able to sufficiently guarantee the capability of chewing, *e.g.* foods, during a whole lifetime it is necessary to keep the teeth in a good condition and to obtain a good oral hygiene. This can be obtained by brushing the teeth frequently using toothpaste or the like. The mouth may further advantageously be rinsed with a mouth wash comprising antimicrobial agents.

To prevent the formation of dental caries, plaque, and tartar, it has been suggested to add a dextranase and/or a mutanase and/or other enzymes to oral care compositions and products.

US patent no. 4,353,891 (Guggenheim et al.) concerns plaque removal using mutanase from *Trichoderma harzianum* CBS 243.71 to degrade mutan synthesized by cultivating *Streptococcus mutans* strain CBS 350.71 identifiable as OMZ 176. It is stated that the critical ingredient in dental plaque is water-insoluble polysaccharide with a-1,3-glucosidic bonds and that such polysaccharide material termed mutan is not attacked by dextranase.

Guggenheim et al., (1972), Caries Res. 6, p. 289-297) discloses that the extent of the dental plaque of rats is not significantly affected by the simultaneous use of a dextranase and a 1,3-glucanase (mutanase).

Hare et al. (1978), Carbohydrate Research 66, p. 245-264, found that a synergistic effect is obtained when hydrolysing and solubilising oral glucans with a bacterial dextranase in combination with bacterial a-1,3 glucanase from *Cladosporium resinae*.

US patent no. 4,438,093 (The Research Foundation for Microbial diseases of Osaka) describes oral compositions comprising a dextranase and a a-1,3-glucanase (mutanase), both being present in an amount of 0.5 to 100 enzyme units per gram of said oral composition, in an enzyme unit ratio of 1:2 to 2:1. Said dextranase is derived from a bacteria within the genus *Coryn-*

bacterium and said a-1,3-glucanase is derived from a bacteria within the genus *Pseudomonas*.

GB 2,206,585 (Dental Chem Co LTD) described a teeth cleaning agent containing hydroxyapatite as polishing agent, with a laevanase, dextranase and mutanase immobilised on the hydroxyapatite.

US patent no. 5,145,665 (Henkel) discloses a composition for the care of the mount and teeth comprising a dextranase and/or a 1,3-glucanase for cleaving polysaccharides in the mouth.

FR 2,651,433 (DANA) concerns dentifrice products containing a dextranase to acts on recent plaque, a mutanase to acts on old and insoluble plaque, and a mixture of other enzymes having bactericide action.

US patent no. 5,320,830 (Proctor & Gamble) describes toothpaste compositions for the reduction of plaque and gingivitis comprising a) a surfactant, b) an enzyme, c) chelating agent d) a fluoride source, e) a silica abrasive and d) a carrier. The enzyme is an endoglucanase, papain, a dextranase and/or a mutanase.

SUMMARY OF THE INVENTION

It is the object of the present invention to provide oral care products which safely (*i.e.* without harming the tissue and structure of the oral cavity) and effectively prevent the formation of dental plaque and/or removes already deposited dental plaque.

The first object of the invention relates to an oral care composition comprising a dextranase and a mutanase both having a significant enzyme activity in the range from pH 4 to below 6.0.

In an embodiment of the invention the dextranase is derived from a strain of the filamentous fungus genus *Paecilomyces* and the mutanase is derived from a strain of *Trichoderma* or *Penicillium*.

In the second aspect the invention relates to an oral care product comprising an oral care composition of the invention.

In an embodiment of the invention the oral care product is a toothpaste comprise a composition described in US patent no. 5,320,830.

In the third aspect the invention relates to the use of a composition of the invention or oral care product of the invention for preventing the formation of dental plaque or removing dental plaque.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the time course of mutan hydrolysis

Figure 2 shows hydrolysis of mutan on glass wall

Figure 3 shows dosage response curve of mutan hydrolysis

Figure 4 shows hydrolysis of mutan with *Paecilomyces lilacinum* dextranase and/or *Trichoderma harzianum* mutanase within the pH range from 5.0 to 8.0.

Figure 5 shows the plaque removing effect of dextranase and a mutanase both having a significant enzyme activity in the range from pH 4 to below 6.0.

DETAILED DESCRIPTION OF THE INVENTION

It is the object of the present invention to provide oral care products which safely (*i.e.* without harming the tissue and structure of the oral cavity) and effectively prevent the formation of dental plaque and/or removes already deposited dental plaque.

It is to be understood that said oral care products directly or indirectly may also have other oral care functions at the same time, *e.g.* prevention of dental holes or gingivitis.

All concerned oral care compositions and products prepared there from referred to in the present application comprise enzymes and have a pH in the range from 4.0 to below 6.0. A suitable examples of such compositions and products are described in US patent no. 5,320,830 (Proctor & Gamble) which is hereby incorporated by reference.

The present inventors have found that it is advantageous, when using a dextranase and a mutanase in oral care products, to use such enzymes having a pH optimum close to the pH of the oral care product in question. This means in the context of the present invention that the pH lies within the range from 4.0 to about below 6.0.

Oral Care Compositions

Accordingly, the first object of the invention is to provide an oral care composition comprising a dextranase and a mutanase both having a significant enzyme activity in the range from pH 4 to below 6.0.

A "significant enzyme activity" means that the relative enzymatic activity of the enzyme is above 70% in particular above 80%, especially above 90% of the activity at the pH optimum.

When using a dextranase and a mutanase having a significant enzymatic activity close to the pH of the oral care product an improved product is obtained, as *e.g.* a less amount of enzyme need to be used to obtain the desired effect and/or less time is need to obtain the desired effect.

The reduced amount of enzyme needed is of commercial interest for oral care product manufactures as the cost of producing such product according to the invention can be reduced. Further, if the original amount of enzymes are added to the product an improved product can be obtained.

Also the user of an oral care product of the invention (which will be describe in more details below), prepared from the oral care composition of the invention, will benefit from the present invention, as the direct and indirect disadvantages (*e.g.* yellow deposits on the teeth and prevention of dental holes and gingivitis, respectively) can be prevented safely and more effectively than with prior art products.

According to the invention all dextranase and mutanases, being of *e.g.* of microbial, such as bacterial or fungi origin, or plant origin, having a pH optimum within pH 4.0 to below 6.0, are contemplated according to the invention and therefor encompassed by the scope of the present invention.

In a specific embodiment of the invention the dextranase may be derived from a strain of the filamentous fungus genus *Paecilomyces*, in particular a strain of *Paecilomyces lilacinum*. *Paecilomyces lilacium* dextranase (available from Novo Nordisk A/S) has a pH optimum at about 5.5.

A mutanase suitable for the use in an oral care composition of the invention may be produced by filamentous fungi from the group including *Trichoderma*, in particular from a strain of *Trichoderma harzianum*, such as *Trichoderma harzianum* CBS 243.71, or *Penicillium*, in particular a strain of *Penicillium funiculosum*, such as *Penicillium funiculosum* NRRL 1768, or a strain of *Penicillium lilacinum*, such as *Penicillium lilacinum* NRRL 896.

As known from US patent 4,353,981 (Guggenheim et al.) the pH optimum of the mutanase produced by *Trichoderma harzianum* CBS 243.71, *Penicillium funiculosum* NRRL 1768 and *Penicillium lilacinum* NRRL 896 have a significant relative enzyme activity (as defined above) within pH 4.0 to below 6.0.

Actually, *Trichoderma harzianum* CBS 243.71 and *Penicillium funiculosum* NRRL 1768 even have a pH optimum within pH 4.0 to below 6.0.

An oral care composition of the invention may suitably have incorporated an amount of dextranase and mutanase equivalent to an enzyme activity, calculated as enzyme activity units in the final oral care product, in the range from 0.001 KDU to 1000 KDU/ml, preferably from 0.01 KDU/ml to 500 KDU/ml, especially from 0.1 KDU/ml to 100 KDU/ml, and from 0.001 MU/ml to 1000 MU/ml, preferably from 0.01 MU/ml to 500 MU/ml, especially from 0.01 MU/ml to 100 MU/ml and from 0.01 MU/ml to 100 MU/ml, respectively.

The present inventors have surprisingly found that when combining a dextranase from *Paecilomyces lilacinum* and a mutanase from *Trichoderma harzianum* a synergistic effect is obtained when hydrolysing mutan at 37°, pH 5.5 (see Example 1).

The hydrolysis of mutan with the combination of said dextranase and mutanase is also increased in comparison with hydrolysis under corresponding conditions using said enzymes separately (see Example 2).

Furthermore, also the dosage response curve of mutan hydrolysis using the above mentioned enzymes (see Example 3) shows a synergistic effect at pH 5.5 at 37°C. This is advantageous as a reduced amount of enzymes need to be used to remove plaque from the teeth or to prevent the formation of plaque on the teeth.

In a preferred embodiment the enzymes used are recombinant.

It is necessary that the enzymes (*i.e.* dextranase and the mutanase) are substantially active at temperatures between 20°C and 45°C, especially around 37°C, as the temperature prevailing in the human mouth lies within said interval.

The term "substantially active" means in the context of the present invention that the enzyme in question has a relative activity above 70%, in particular above 80%, especially above 90% of the activity at the temperature optimum.

It is also contemplated according to the invention to include other enzyme activities in the oral care compositions of the invention. Contemplated enzymes, beside dextranase and mutanase, may be from the group including proteases, such as papain, endoglucosidases, lipases, amylase and mixtures thereof.

Oral care products

The invention also relates to oral care products comprising an oral care composition of the invention. The oral care product may have any suitable physical form (*i.e.* powder, paste, gel, liquid, ointment, tablet etc.). An "oral care product" can be defined as a product which can be used for maintaining or improving the oral hygiene in the mouth of humans and animals, by preventing formation of dental plaque, removing dental plaque, preventing and/or treating dental diseases etc.

At least in the context of the present invention oral care products do also encompass products for cleaning dentures, artificial teeth and the like.

Examples of such oral care products include toothpaste, dental cream, gel or tooth powder, odontic, mouth washes, pre- or post brushing rinse formulations, chewing gum, lozenges, and candy.

Toothpastes and tooth gels typically include abrasive polishing materials, foaming agents, flavouring agents, humectants, binders, thickeners, sweetening agents, whitening/bleaching/ stain removing agents, water, and optionally enzymes.

Mouth washes, including plaque removing liquids, typically comprise a water/alcohol solution, flavour, humectant, sweetener, foaming agent, colorant, and optionally enzymes.

Abrasive polishing material might also be incorporated into the dentifrice product of the invention. According to the invention said abrasive polishing material includes alumina and hydrates thereof, such as alpha alumina trihydrate, magnesium trisilicate, magnesium carbonate, kaolin, aluminosilicates, such as calcined aluminum silicate and aluminum silicate, calcium carbonate, zirconium silicate, and also powdered plas-

tics, such as polyvinyl chloride, polyamides, polymethyl methacrylate, polystyrene, phenol-formaldehyde resins, melamine-formaldehyde resins, urea-formaldehyde resins, epoxy resins, powdered polyethylene, silica xerogels, hydrogels and aerogels and the like. Also suitable as abrasive agents are calcium pyrophosphate, water-insoluble alkali metaphosphates, dicalcium phosphate and/or its dihydrate, dicalcium orthophosphate, tricalcium phosphate, particulate hydroxyapatite and the like. It is also possible to employ mixtures of these substances.

Dependent on the oral care product the abrasive product may be present in from 0 to 70% by weight, preferably from 1% to 70%. For toothpastes the abrasive material content typically lies in the range of from 10% to 70% by weight of the final toothpaste product.

Humectants are employed to prevent loss of water from *e.g.* toothpastes. Suitable humectants for use in oral care products according to the invention include the following compounds and mixtures thereof: glycerol, polyol, sorbitol, polyethylene glycols (PEG), propylene glycol, 1,3-propanediol, 1,4-butanediol, hydrogenated partially hydrolysed polysaccharides and the like. Humectants are in general present in from 0% to 80%, preferably 5 to 70% by weight in toothpaste.

Silica, starch, tragacanth gum, xanthan gum, extracts of Irish moss, alginates, pectin, cellulose derivatives, such as hydroxyethyl cellulose, sodium carboxymethyl cellulose and hydroxypropyl cellulose, polyacrylic acid and its salts, polyvinylpyrrolidone, can be mentioned as examples of suitable thickeners and binders, which helps stabilizing the dentifrice product. Thickeners may be present in toothpaste creams and gels in an amount of from 0.1 to 20% by weight, and binders to the extent of from 0.01 to 10% by weight of the final product.

As foaming agent soap, anionic, cationic, non-ionic, amphoteric and/or zwitterionic surfactants can be used. These may be present at levels of from 0% to 15%, preferably from 0.1 to 13%, more preferably from 0.25 to 10% by weight of the final product.

Surfactants are only suitable to the extent that they do not exert an inactivation effect on the present protease. Surfactants include fatty alcohol sulphates, salts of sulphonated mono-glycerides or fatty acids having 10 to 20 carbon atoms, fatty acid-albumen condensation products, salts of fatty acids amides and taurines and/or salts of fatty acid esters of isethionic acid.

Suitable sweeteners include saccharin.

Flavours, such as spearmint, are usually present in low amounts, such as from 0.01% to about 5% by weight, especially from 0.1% to 5%.

Whitening/bleaching agents include H_2O_2 and may be added in amounts less that 5%, preferably from 0.25 to 4%, calculated on the basis of the weight of the final product.

Water is usually added in an amount giving *e.g.* toothpaste a flowable form.

Further water-soluble anti-bacterial agents, such as chlorhexidine digluconate, hexetidine, alexidine, quaternary ammonium anti-bacterial compounds and water-soluble sources of certain metal ions such as zinc, copper, silver and stannous (*e.g.* zinc, copper and stannous chloride, and silver nitrate) may also be included.

Also contemplated according to the invention is the addition of compounds which can be used as fluoride source, dyes/colorants, preservatives, vitamins, pH-adjusting agents, anti-caries agents, desensitizing agents etc.

Other essential components used in oral care products and in oral care products of the invention are enzymes. Enzymes are biological catalysts of chemical reactions in living systems. Enzymes combine with the substrates on which they act forming an intermediate enzyme-substrate complex. This complex is then converted to a reaction product and a liberated enzyme which continue its specific enzymatic function.

Enzymes provide several benefits when used for cleansing of the oral cavity. Proteases break down salivary proteins, which are adsorbed onto the tooth surface and form the pellicle, the first layer of resulting plaque. Proteases along with lipases

destroy bacteria by lysing proteins and lipids which form the structural components of bacterial cell walls and membranes. Dextranase breaks down the organic skeletal structure produced by bacteria that forms a matrix for bacterial adhesion. Proteases and amylases, not only prevents plaque formation, but also prevents the development of calculus by breaking-up the carbohydrate-protein complex that binds calcium, preventing mineralization.

A toothpaste produced from an oral care composition of the invention (in weight % of the final toothpaste composition) may typically comprise the following ingredients:

Abrasive material	10 to 70%
Humectant	0 to 80%
Thickener	0.1 to 20%
Binder	0.01 to 10%
Sweetener	0.1% to 5%
Foaming agent	0 to 15%
Whitener	0 to 5%
Enzymes	0.0001% to 20%

In a specific embodiment of the invention the oral care product is toothpaste having a pH in the range from 4.0 to about below 6.0 comprising

a) 10% to 70%	Abrasive material
b) 0 to 80%	Humectant
c) 0.1 to 20%	Thickener
d) 0.01 to 10%	Binder
e) 0.1% to 5%	Sweetener
f) 0 to 15%	Foaming agent
g) 0 to 5%	Whitener
i) 0.0001% to 20%	Enzymes.

Said enzymes referred to under i) include dextranase and mutanase described above, and optionally other types of enzymes mentioned above known to be used in toothpastes and the like.

In an embodiment of the invention the oral care product is a toothpaste have any of the composition encompassed by US patent no. 5,320,830 (from Proctor & Gamble).

A mouth wash produced from an oral care composition of the invention (in weight % of the final mouth wash composition) may typically comprise the following ingredients:

0-20%	Humectant
0-2%	Surfactant
0-5%	Enzymes
0-20%	Ethanol
0-2%	Other ingredients (*e.g.* flavour, sweetener active ingredients such as fluorides).
0-70%	Water

The mouth wash composition may be buffered with an appropriate buffer to pH 4 to below 6.

The mouth wash may be in none-diluted form (*i.e.* must be diluted before use).

Said enzymes referred include a dextranase and mutanase described above, and optionally other types of enzymes mentioned above known to be used in mouth washes.

Use of an Oral Care Composition or Product

In the third aspect the invention relates to the use of the composition of the invention or an oral care product of the invention for preventing the formation of plaque or for removing dental plaque.

Using a product of the invention typically involves applying a safe and effective amount of said product to the oral cavity. These amounts (*e.g.* from 0.3 to about 2 grams), if it is a toothpaste or toothgel, is kept in the mount from about 15 seconds to about 12 hours.

Method of Manufacture

The oral care composition and products of the present invention can be made using methods which are common in the oral product area.

MATERIALS AND METHODS

Materials

Dextranase produced by *Paecilomyces lilacinum* (available from Novo Nordisk A/S).

Mutanase produced by *Trichoderma harzianum* CBS 243.71 (available from Novo Nordisk A/S)

Microorganisms:

Streptococcus mutans strain *CBS 350.71* (or OMZ 176)

Actinomyces viscosus DSM 43329

Fusobacterium nucleatum subsp. polymorphum DSM 20482

Solutions

Britton-Robinson Buffer

Erythrosin B (Sigma)

Equipment

Shaker (Eppndorf Thermomixer, Type 5436)

Chromameter CR-200 (Minolta).

Preparation of hydroxyapatite disks

Hydroxyapatite disks are prepared by compressing 250 mg of hydroxyapatite in a disk die at about 5,900 kg (13,000 lbs) of pressure for 5 minutes. The disks are then sintered at 600°C for 4 hours and finally hydrated with sterile de-ionised water.

Sterilisation of hydroxyapatite disks

HA disks are sterilised at 180°C for two hours, hydrated with the sterilised de-ionised water and placed in a lid of Nunc tube (10 ml volume).

Preparation of Mutan

Mutan is prepared by growing *Streptococcus mutans* CBS 350.71 at pH 6.5, 37°C (kept constant), and with an aeration rate of 75 rpm in a medium comprised of the following components:

NZ-Case	6.5 g/liter
Yeast Extract	6 g/liter
$(NH_4)_2SO_4$	20 g/liter
K_2PO_4	3 g/liter
Glucose	50 g/liter
Pluronic PE6100	0.1 %

After 35 hours, sucrose is added to a final concentration of 60 g/liter to induce glucosyltransferase. The total fermentation time is 75 hours. The supernatant from the fermentation is centrifuged and filtered (sterile). Sucrose is then added to the supernatant to a final concentration of 5 % (pH is adjusted to pH 7.0 with acetic acid) and the solution is stirred overnight at 37°C. The solution is filtered and the insoluble mutan is harvested on propex and washed extensively with deionized water containing 1% sodium benzoate, pH 5 (adjusted with acetic acid). Finally, the insoluble mutan is lyophilized and ground.

Determination of dextranase activity (KDU)

One Kilo Novo Dextranase Unit (1 KDU) is the amount of enzyme which breaks down dextran forming reducing sugar equivalent to 1 g maltose per hour in Novo Nordisk' method for determination of dextranase based on the following standard conditions:

Substrate.....Dextran 500 (Pharmacia)
Reaction time.....20 minutes
Temperature.....40°C
pH.....5.4

A detailed description of Novo Nordisk's analytical method (AF 120) is available on request.

Determination of mutanase activity (MU)

One Mutanase Unit (MU) is the amount of enzyme which under standard conditions liberates 1 mmol reducing sugar (calculated as glucose) per minute.

Standard Conditions

Substrate.....1.5% mutan

Reaction time.....15 minutes
Temperature.....40°C
pH.....5.5
A detailed description of Novo Nordisk's analytical method (AF 180/1-GB) is available from Novo Nordisk A/S on request.

Preparation of mutan adhered glass wall

Streptococcus mutans OMZ 176 (CBS 350.71) is inoculated in a glass tube (22 mm diameter x 150 mm height) containing 10 ml Todd Hewitt Broth with 2% sucrose and the tube is allowed to stand overnight at 37°C. The broth is discarded and adhered mutan and *Streptococcus mutans* cells on glass wall are washed twice with 10 ml of 0.85% NaCl solution.

Assessment of the plaque removing effect

The method used for assessing the plaque removal effect is based on the method described by Kao in JP2250816. According to the present method the hydroxyapatite disks are coated with a biofilm comprising three strain of oral micro-organisms (*Streprtococcus mutans, Actinomyces viscosus* and *Fusobacterium nucleatum)*.

To test the plaque removing effect 0.1 % Erythrosin B in PBS is used to stain plaque present on the hydroxyapatite disks red. The intensity of the red color (*i.e.* a*) is measured on a Chromameter CR-200. The max. a* value is 60. Values below that indicate a less intensive red color (*i.e.* less plaque present). If the a* value is determined to zero no red color is present (*i.e.* no plaque).

EXAMPLES

EXAMPLES

Example 1

Time Course of Mutan Hydrolysis

Mutan prepared as described in the "Materials and Method" section was dispersed in deionized water with an ultrasonicater in a concentration of 16 mg/ml to prepare a substrate suspension.

Paecilomyces lilacinum mutanase and *Trichoderma harzianum* CBS 243.71 dextranase dissolved in a 0.05 M acetate buffer were diluted with deionized water.

The following enzyme solutions were prepared:

- Dextranase solution (4 KDU/ml),
- Mutanase solution (4 MU/ml), and
- a mixed Dextranase and Mutanase solution (4 KDU and 4 MU/ml).

50 mM Britton-Robinson buffer solutions having the pH adjusted to 5.5 were also prepared.

250 ml of each of the above mentioned enzyme solutions and 500 ml of buffer solution (pH 5.5) were mixed in a microcentrifuge tube. Immediately thereafter 250 ml of Mutan suspension was added, incubated at 37°C in a shaker at the maximum speed.

After exactly 30 and 60 minutes, 250 ml of a 0.5 N HCl was added to terminate the enzymatic reaction. A 0 time control was prepared by adding a 0.5 N HCl before addition of a substrate suspension. Each of the reaction mixtures were subjected to centrifugation. The solubilized sugar, in the obtained supernatant, was determined according to the anthrone reaction method (J.H. Roe, (1955), J. Biol. Chem. **212**, p. 335).

The results are illustrated in Figure 1. From Figure 1 it can be seen that the combined use of the above mentioned Dextranse and Mutanase give a synergistic effect when hydrolysing mutan at pH 5.5.

Example 2

Hydrolysis of Mutan adhered to glass walls

To mimic dental plaque adhering on the teeth surface glass tubes with adhered mutan were prepared as described above in

the "Material and Methods"-section. Further, the following enzyme solutions were prepared as described in Example 1:

- Dextranase solution (1 KDU/ml),
- Mutanase solution (1 MU/ml), and
- a mixed Dextranase and Mutanase solution (1 KDU and 1 MU/ml).

50 mM Britton-Robinson buffer solutions having the pH adjusted to 5.5 were also prepared.

5 ml of the above enzyme solutions, incubated at 37°C for 15 minutes prior to the experiment, was poured to the glass tube with the adhered mutan. The glass tubes were incubated at 37°C.

1000 ml of a 0 time control was taken out immediately and mixed with 500 ml of 0.5 N HCl to stop the enzymatic reaction. At 5, 10, 15, 30 minutes, 1000 ml samples were taken out and immediately mixed with 500 ml of 0.5 N HCl. Each of the reaction mixture was subjected to centrifugation. The solubilized sugar in the obtained supernatant was determined in the same manner as Example 1.

The result of the experiments are displayed in Figure 2. As can be seen from Figure 2 the combined use of the Dextranase and the Mutanase give a synergistic effect when hydrolysing mutan adhered on a glass wall at pH 5.5.

Example 3

Effect of Dextranase and Mutanase activity

A Mutan suspension was prepared in the same manner as Example 1.

The following Mutanase and Dextranase solutions dissolved in a 0.05 M acetate buffer were prepared a described in Example 1:

- Dextranase solution (0.4, 2.0 and 4.0 KDU/ml),
- Mutanase solution (0.4, 2.0 and 4.0 MU/ml), and
- a mixed enzyme solution (0.4, 2.0 and 4.0 KDU and 0.4, 2.0 and 4.0 MU/ml, respectively).

250 ml of the above enzyme solutions and 500 ml of 0.05 M acetate buffer (pH 5.5) were mixed in a microcentrifuge tube.

Immediately thereafter 250 ml of the prepared Mutan suspension was added, incubated at 37°C in a shaker at maximum speed.

After exactly 60 minutes, 250 ml of 0.5 N HCl was added to terminate the enzymatic reaction. A 0.5 M acetate buffer solution (pH 5.5) was applied instead of an enzyme solution as a control. Each of the reaction mixture was subjected to centrifugation. The solubilized sugar in the obtained supernatant was determined in the same manner as Example 1.

The result of the experiments are displayed in Figure 3. As can be seen from Figure 3 the combined use of the Dextranase and the Mutanase give as a synergistic effect when hydrolysing mutan at pH 5.5.

Example 4

Hydrolysis of Mutan at pHs from 5.0 to 8.0

A Mutan suspension was prepared in the same manner as Example 1.

The following Mutanase and Dextranase solutions dissolved in a 0.05 M acetate buffer were prepared a described in Example 1:

- Dextranase solution (4 KDU/ml),
- Mutanase solution (4 MU/ml), and
- a mixed enzyme solution (4 KDU and 4 MU/ml).

Further, 50 mM Britton-Robinson buffer solutions having the pH adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were also prepared.

Then 250 ml of enzyme solution and 500 ml of buffer solution were mixed in a microcentrifuge tube. Immediately thereafter 250 ml of the prepared Mutan suspension was added and incubated at 37 °C in a shaker at the maximum speed. After exactly 30 minutes, 250 ml of 0.5 N HCl was added to terminate the enzymatic reaction. Each of the reaction mixtures were subjected to centrifugation.

The solubilized sugar in the obtained supernatant was determined in the same manner as Example 1.

The result of the experiments are displayed in Figure 4.

As can be seen from Figure 4 the combined use of the Dextranase and the Mutanase give a synergistic effect within pHs from 5.0 to 8.0.

Example 5

Plaque removing effect at pH 5.5

Three oral microorganisms, *Streprtococcus mutans, Actinomyces viscosus* and *Fusobacterium nucleatum, respectively,* were cultivated anaerobically for three days at 37 °C. Hydroxyapatite disks coated with sterilized saliva were immersed in a culture broth during cultivation so that oral biofilm was formed on a salivary coated hydroxyapatite disks. After cultivation, the disks were briefly rinsed with a phosphate buffered saline and then treated with the enzyme solution prepared in 40 mM Britton-Robinson buffer pH 5.5 shown in a Table for 20 minutes at 37°C.

Table 1

Treatment	Mutanase	Dextranase
1	0	0
2	1.5 MU/ml	0
3	0	1.5 kDU/ml
4	1.5 MU/ml	1.5 kDU/ml

The disks were rinsed briefly with PBS and then incubated in a 1 ml 0.1 % Erythrosin B (Sigma) in PBS for 1 minute to stains plaque present on the hydroxyapatite disks red. The disks were air dried overnight. The intensity of the red color (*i.e.* a*) was measured on a Chromameter CR-200. The higher the a* value is the more red are the hydroxyapatite disks. The Erythrosin B solution was removed and the disks were rinsed with PBS for a few minutes.

The result of the test is shown in Figure 5. As can be seen hydroxyapatite disk treated with 1.5 KDU/ml dextranase and 1.5 MU/ml mutanase removes plaque more efficiently than dextranase and mutanase separately. The combination of

dextranase and mutanase has a synergistic effect on the plaque removal effect at pH 5.5.

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

PATENT CLAIMS

1. An oral care composition comprising a dextranase and a mutanase both having a significant enzyme activity in the range from pH 4 to below 6.0.

2. The oral care composition according to claim 1, wherein the activity of the dextranase and the mutanase are above 70%, in particular above 80%, especially above 90%, even better 100% of the relative enzyme activity at the pH optimum.

3. The oral care composition according to claims 1 and 2, wherein the dextranase is derived from *Paecilomyces*, in particular *Paecilomyces lilacinum*.

4. The oral care composition according to claims 1 to 3, wherein the mutanase is derived from *Trichoderma*, in particular *Trichoderma harzianum*, *Trichoderma harzianum* CBS 243.71, or *Penicillium*, in particular *Penicillium funiculosum*, especially *Penicillium funiculosum* NRRL 1768, or *Penicillium lilacinum*, especially *Penicillium lilacinum* NRRL 896.

5. The oral care composition of claim 4, wherein the mutanase is recombinant.

6. The oral care composition according to any of claims 1 to 5, wherein the enzymes is substantially active in the composition at temperatures between 20°C and 40°C, especially around 37°C.

7. The oral care composition according to any of claims 1 to 6, further comprising an enzyme selected from the group of proteases, such as papain, endoglucosidase, lipase, amylase, and mixtures thereof.

8. An oral care product comprising an oral care composition of any of claims 1 to 7.

9. The oral care product according to claim 8, being a dentifrice, such as a toothpaste, tooth powder or a mouth wash.

10. The oral care product according to claim 9, wherein the product is a toothpaste or mouth wash having a pH in the range from 4.0 to about below 8.

11. The oral care product according to claim 10, wherein the tooth paste comprise a composition described in US patent no. 5,320,830.

12. Use of a composition of claims 1 to 7 or oral care product according to claims 8 to 11 for preventing the formation of dental plaque or removing dental plaque.

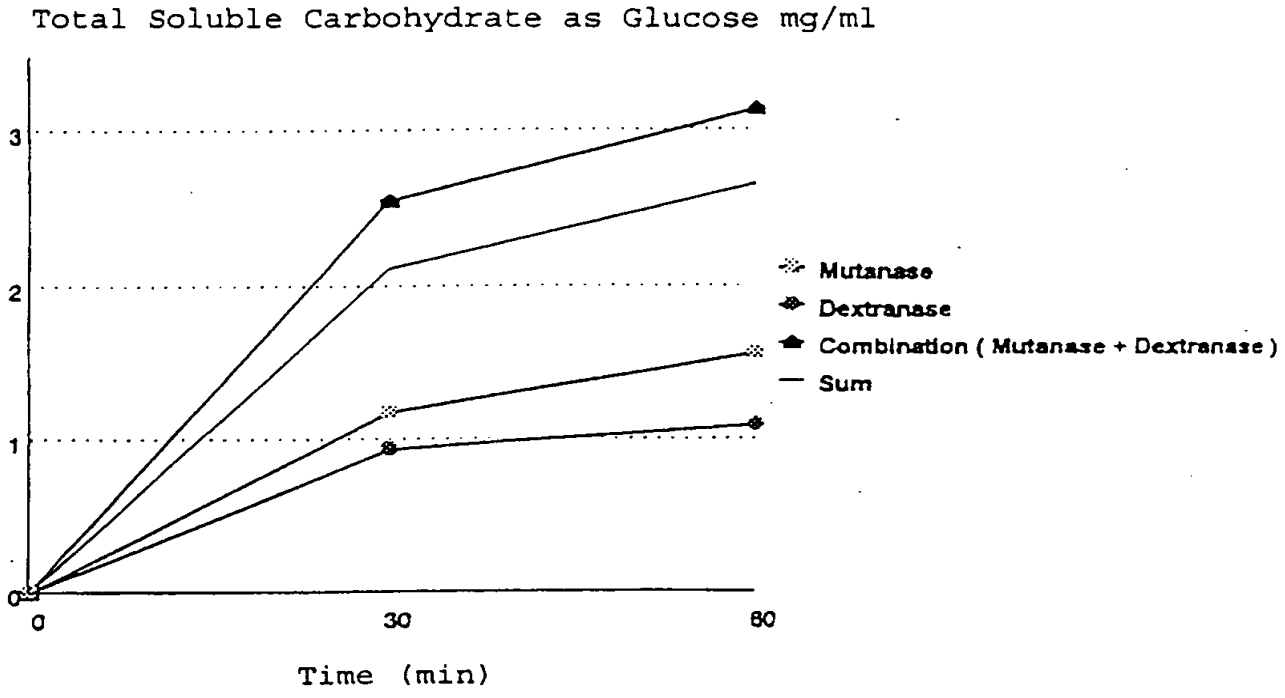
Total Soluble Carbohydrate as Glucose mg/ml
3
2
1
0
0
30
60
Time (min)
Mutanase
Dextranase
Combination (Mutanase + Dextranase)
Sum

Fig. 1

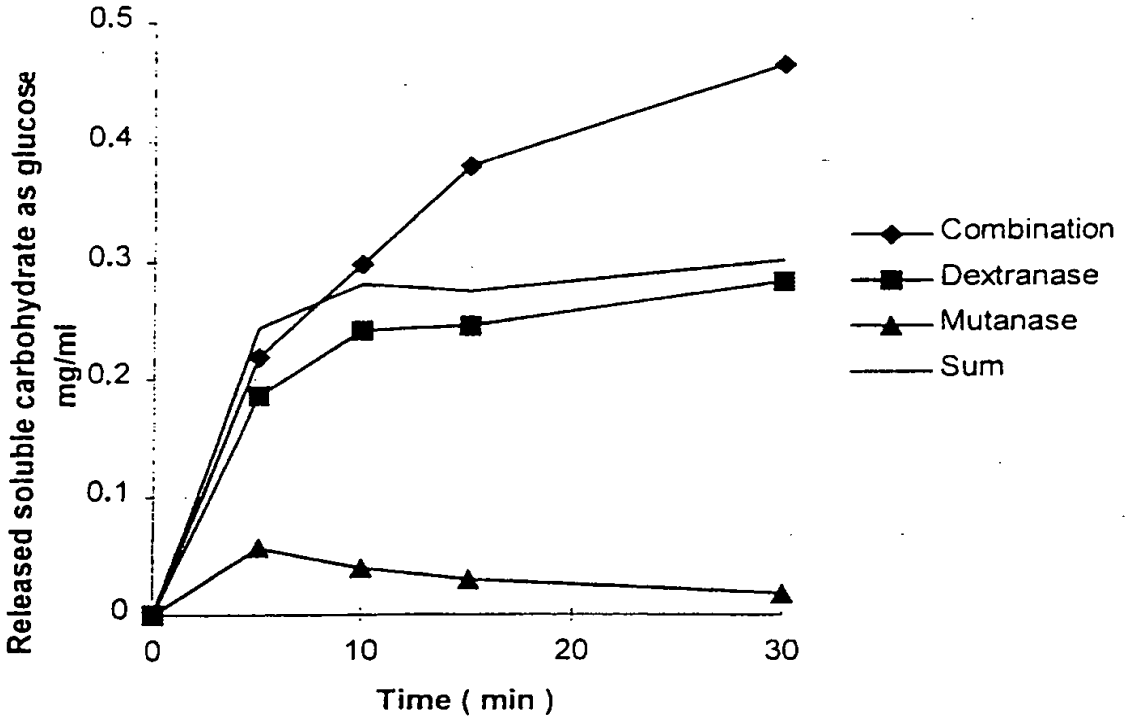
Released soluble carbohydrate as glucose mg/ml
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
Time (min)
Combination
Dextranase
Mutanase
Sum

Fig. 2

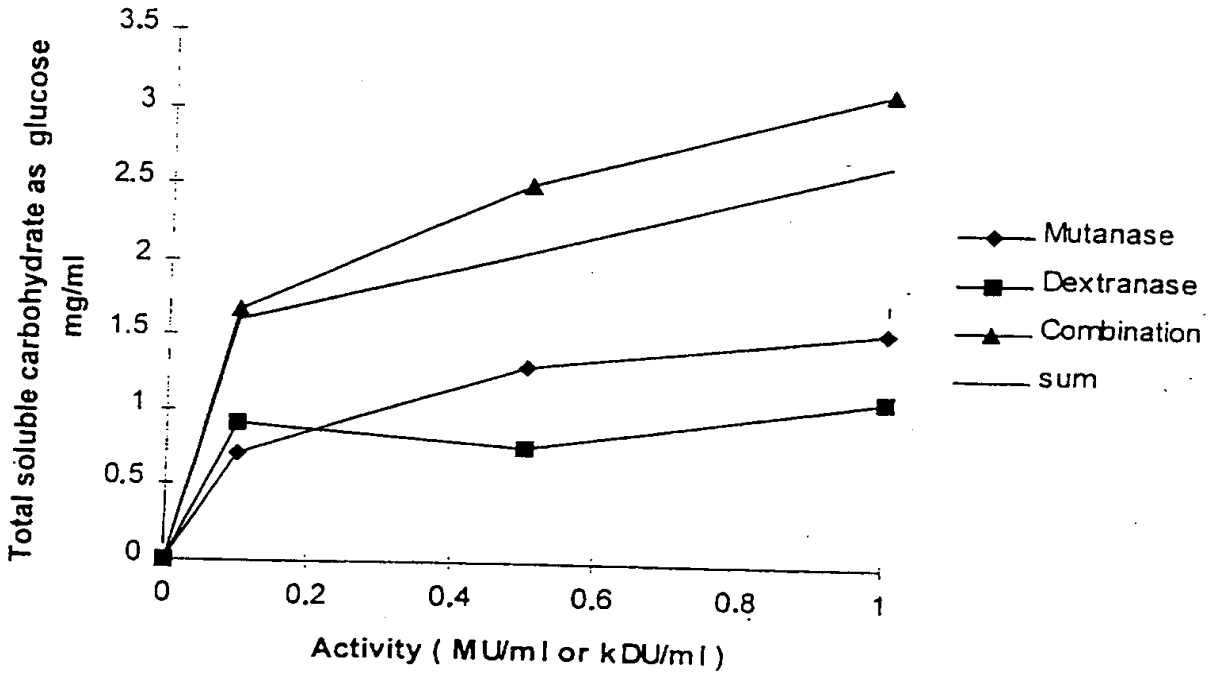
Total soluble carbohydrate as glucose mg/ml
3.5
3
2.5
2
1.5
1
0.5
0
0
0.2
0.4
0.6
0.8
1
Activity (MU/ml or kDU/ml)
Mutanase
Dextranase
Combination
sum

Fig. 3

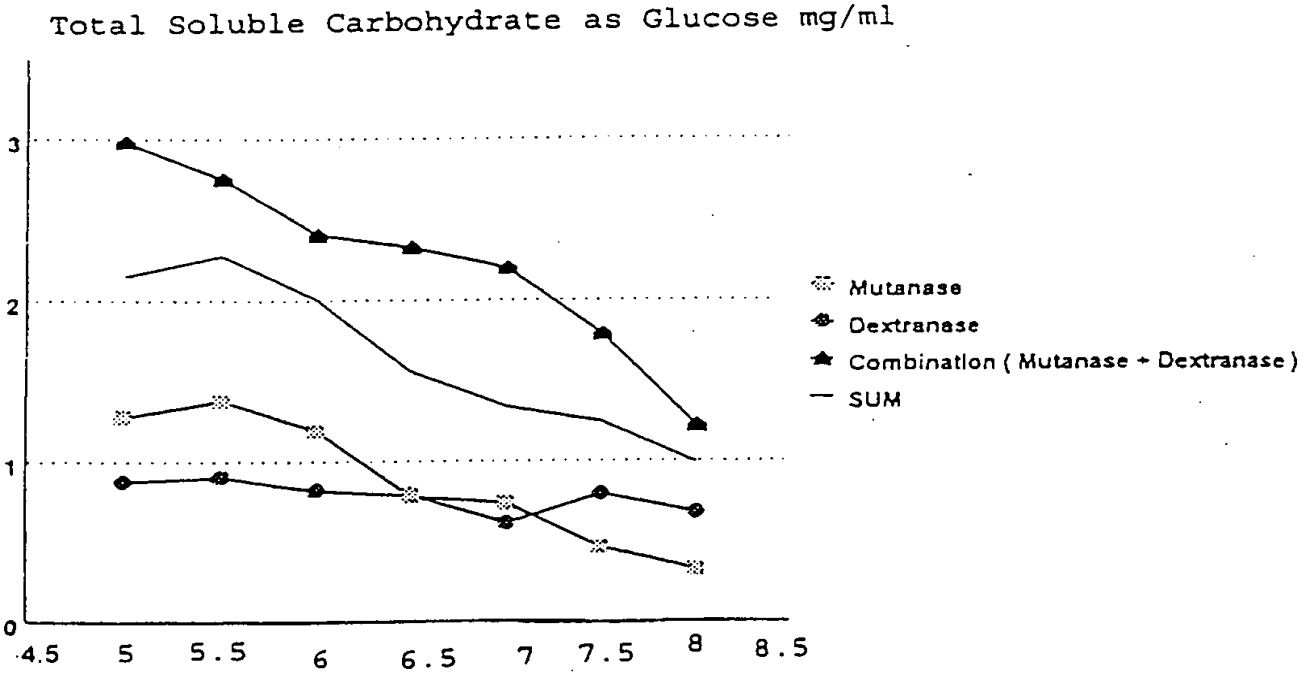
Total Soluble Carbohydrate as Glucose mg/ml
3
2
1
0
4.5
5
5.5
6
6.5
7
7.5
8
8.5
Mutanase
Dextranase
Combination (Mutanase + Dextranase)
SUM

Fig. 4

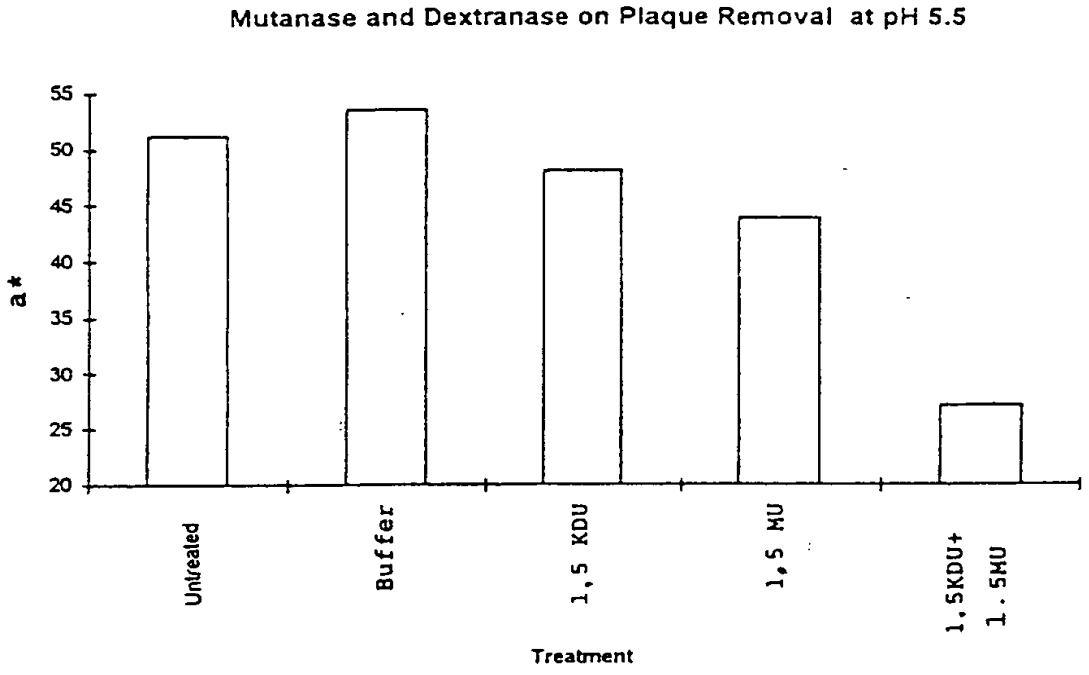
Mutanase and Dextranase on Plaque Removal at pH 5.5
a*
55
50
45
40
35
30
25
20
Untreated
Buffer
1,5 KDU
1,5 MU
1,5KDU+ 1.5MU
Treatment

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00163

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 7/28, A61K 38/47
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, WPI, DIALINDEX (ALLSCIENCE)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5320830 A (MICHAEL F. LUKACOVIC ET AL), 14 June 1994 (14.06.94), claim 3 --	1-12
X	US 4466954 A (HIROMICHI ICHIKAWA ET AL), 21 August 1984 (21.08.84), column 1, line 66; column 6, line 16 - line 21 --	1-12
X	Dialog Information Services, File 351, Dialog accession no. 004561593, WPI accession no. 86-064937/198610, Lion Corp: "Tooth paste compsn.- comprising reformed aluminium hydroxide and enzyme(s)", JP,A,61015827, 19860123 --	1-12

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 July 1997	25 -07- 1997
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00163

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, file 155, Medline, Dialog accession no. 02909591, Medline accession no. 77150500, Budtz-Jorgensen E. et al: "Enzymes as denture cleansers", Scand J Dent Res. (DENMARK) Mar 1977, 85 (3) p209-15 --	1-12
A	File WPI, Derwent accession no. 72-56480T, Kojin Co Ltd: "Extranase prodn - used in prevention of caries", JP,B,47034148B, DW7235 -- --------	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/07/97

International application No.
PCT/DK 97/00163

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5320830 A	14/06/94	AU 5804194 A	15/08/94
		BR 9307801 A	14/11/95
		CA 2151935 A	21/07/94
		CN 1095586 A	30/11/94
		CZ 9501735 A	13/12/95
		EP 0676950 A	18/10/95
		HU 72472 A	29/04/96
		HU 9501950 D	00/00/00
		JP 8505390 T	11/06/96
		US 5437856 A	01/08/95
		WO 9415579 A	21/07/94
US 4466954 A	21/08/84	DE 3248541 A,C	07/07/83
		FR 2518883 A,B	01/07/83
		GB 2112284 A,B	20/07/83
		HK 72486 A	03/10/86
		JP 1495395 C	16/05/89
		JP 58118509 A	14/07/83
		JP 62034013 B	24/07/87